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Targeting fibrin in neurodegeneration

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Deposition of fibrin in the brain and central nervous system occurs upon injury or disease. This process unmasks a conserved cryptic epitope of fibrin that activates microglia; blocking this interaction can limit inflammation and neurotoxicity.

The clotting factor fibrin plays a crucial role in blood coagulation. Akassoglou and colleagues define a novel specific role of fibrin in neurodegeneration[1], where important unmet medical needs exist for neurodegenerative and neuroinflammatory diseases like Alzheimer's disease (AD) and multiple sclerosis (MS). Only symptomatic therapies are available for AD, and, although MS can be treated with several immunomodulatory drugs, there is no remedy for the processes that drive nerve cell damage, demyelination and clinical disability at the later stages, most notably radical-mediated stres[2]. Akassoglou and colleagues[1] provide an elegant approach that could lead into an urgently needed disease-modifying therapy for both AD and MS[2].

The solution of the Akassoglou group and collaborators derives from a long interest in deciphering how factors of the coagulation system contribute to a range of pathologies in the brain[2]. Blood clotting involves a series of well-orchestrated cellular and molecular steps that lead to temporally closing a ruptured vessel and, starting from blood components contacting the injured vessel endothelium, changes in platelets, and activation of the so-called extrinsic pathway of the coagulation cascade. The latter refers to activation of several enzymes beginning with prothrombin, which is produced in the liver and activated to thrombin by tissue factor. The following intermediate steps lead to activation of coagulation factor X and then cleavage of the plasma protein fibrinogen to fibrin. The final common pathway results in closing the damaged vessel by a polymerized fibrin clot.

Physiological functions of coagulation products are, however, not only clotting, but also trapping bacteria, direct antimicrobial effects, tissue remodeling, and innate immune activation[3-5]. These functions have received overall less attention. Yet another facet of the coagulation system has been brought to our attention by the work of Akassoglou and others[5–7]. It was already observed several decades ago that fibrin deposition frequently occurs in the brain tissue of patients suffering from neurodegenerative and neuroinflammatory conditions such as AD and MS[6,7], but also in the experimental model of the latter, experimental autoimmune encephalomyelitis (EAE)[8]. The damaging role of fibrin(ogen) leakage in these conditions was underscored by the tight relationship of tissue fibrin and clinical severity of EAE, worsening EAE when the gene encoding tissue plasminogen activator (tPA) is deleted, and several other studies. Previous studies over three decades ago showed that fibrin deposition can be targeted by a synthetic inhibitor of plasminogen activator[9].

It is now clear that the vascular system and brain engage in intense crosstalk[4,5]. Beyond supplying blood and oxygen cerebrovascular interactions are involved in maintaining endothelial cell homeostasis, synaptic activity and neurogenesis in the adult organism among others. Vessel damage on the other hand is involved in a host of pathologies ranging from bacterial meningitis to glioblastoma and AD. Whenever blood components leak into brain tissue, fibrin deposition then interacts through receptor-ligand interactions and signaling steps involving MAPK and NF-κB with resident microglia and invading immune cells. Immune cell-derived cytokines like tumor necrosis factor (TNF) enhance fibrin formation and coagulation[5].

The study by Akassoglou and colleagues in this issue expands existing knowledge and lays the ground for a new therapeutic strategy[1]. It shows that fibrin interacts via a cryptic epitope, fibrin γ377-395, which is only exposed after fibrin polymerization, with the CD11b I-domain of complement receptor 3 (CR3) of microglia and macrophages and activates these innate immune cells (Fig. 1). They reasoned that a monoclonal antibody (mAb) that binds to this cryptic epitope, but not the γ400-411 site of fibrin, which mediates fibrin polymerization, should affect innate immune activation but not coagulation. By screening mAbs for differential binding to γ377-395, but not the γ400-411 epitope and another cryptic epitope, they identified mAb 5B8, which fulfils these criteria and binds with high affinity to the γ377-395 site only. Subsequent transcriptomics experiments demonstrate that fibrin binding to CR3 results in upregulation of a number of proinflammatory genes, among them *Ncf4,* an important component of the NADPH oxidase (Nox2) complex, which has been

implicated in stroke and neurodegenerative diseases[10]. 5B8 inhibits microglia activation and the NADP oxidase pathway and prevents the release of reactive oxygen species (ROS). The potential neuroprotective effects were confirmed in co-cultures of fibrin-activated macrophages and neurons, where 5B8 reduced the damage and loss of neurons. These data were corroborated by testing 5B8 in three EAE models. 5B8 reduced the clinical signs and inhibited the accumulation of microglia and infiltration of macrophages in the spinal cord, but did not affect peripheral adaptive immune responses.

Finally, Akassoglou and colleagues report the therapeutic potential of 5B8 in a model of AD[1], the 5XFAD mouse, in which AD-mutant forms of human amyloid precursor protein and presenilin are overexpressed in neurons. Fibrin deposition in the brain of 5XFAD mice progressively accumulates from 3 months onward. Systemic administration of 5B8 interestingly reaches the brain and sites of plaque formation and reduces both microglia activation and the loss of cholinergic neurons, a hallmark of AD. Transcriptome analysis of the brain cortex in 5B8-treated 5XFAD mice demonstrated that 5B8 preferentially suppresses five immune pathways, most notably a hub of genes belonging to the complement pathway, i.e. *Tyrobp* (also known as *Dap12*)*, C4b and C1q*. *Tyrobp* codes for a coreceptor of CD11b and TREM2, another molecule that has been implicated in AD[11].

What are the possible implications of these observations? In MS, selectively targeting oxidative stress and the so-called neurodegenerative aspects by 5B8 appears very attractive, since there is no therapy for these yet. Similarly in AD, complement factors are not only among the major risk genes[12], but fibrin deposition and innate immune activation at the sites of amyloid plaques are considered important contributors to the progressive loss of nerve cells. So far, only the symptoms of AD can be treated by improving cholinergic nerve function, and while many compounds are in development, several have failed already and none directly targets the fibrin-mediated immune activation. The fact that 5B8 penetrates the blood-brain-barrier, does not affect blood clotting, and leaves other pathways of innate immune activation and adaptive immunity seemingly intact, while inhibiting a core pathomechanism in MS, AD and other (neuro)inflammatory conditions such as stroke and rheumatoid arthritis, indicate that it could be a very interesting and novel approach to treat these diseases. Due to the many differences between rodents and humans, for example with respect to immune function[13], a note of caution is warranted, and the data should be validated in human systems. Furthermore, interactions of a fibrin γC peptide truncated at position 399 have been shown to inhibit T cell activation[14] and, on the other hand, fibrin aids in the host defense against bacteria via both innate and T cell-

mediated mechanisms[15]. Targeting a specific site of fibrin that separates different functions of this pleiotropic mediator therefore holds great promise, but the effects of 5B8 could be even broader than indicated by the study of Akassoglou and colleagues[1] and should be followed carefully during clinical development for effects and adverse events.

References

1. Ryu, K.R., *et al. Nat. Immunol.* **19**, XXX–XXX (2018).

2. Lassmann, H., van Horssen, J. & Mahad, D.. *Rev. Neurol.* **8**, 647–656 (2012).

3. Colvin, R.B. & Dvorak, H.F. *J. Exp. Med.* **142**, 1377–1390 (1975).

4. Melchor, J.P. & Strickland, S. *Thromb. Hemost.* **93**, 655–660 (2005).

5. Adams, R.A., Passino, M., Sachs, B.D., Nuriel, T. & Akassoglou, K. *Mol . Interv.* **4**, 163–176 (2004).

6. Cortes-Canteli, M., Zamolodchikov, D., Ahn, H.J., Strickland, S. & Norris, E.H. *J. Alzheimers. Dis.* **32**, 599–608 (2012).

7. Sobel, R. & Mitchell, M.E. *Am. J. Pathol.* **135**, 161–168 (1989).

8. Satoh, J. *et al. J. Immunol.* **138**, 179-184 (1987).

9. Koh, C.-S. & Paterson, P.Y. *Cell. Immunol.* **107**, 52–63 (1987).

10. Ansari, M.A. & Scheff, S.W. *J. Neuropathol. Exp. Neurol.* **69**, 155–167 (2010).

11. Sims, R. *et al*. *Nat .Genet.* **49**, 1373–1384 (2017).

12. Cuyvers, E. & Sleegers, K. *Lancet Neurol.* **15**, 857–868 (2016).

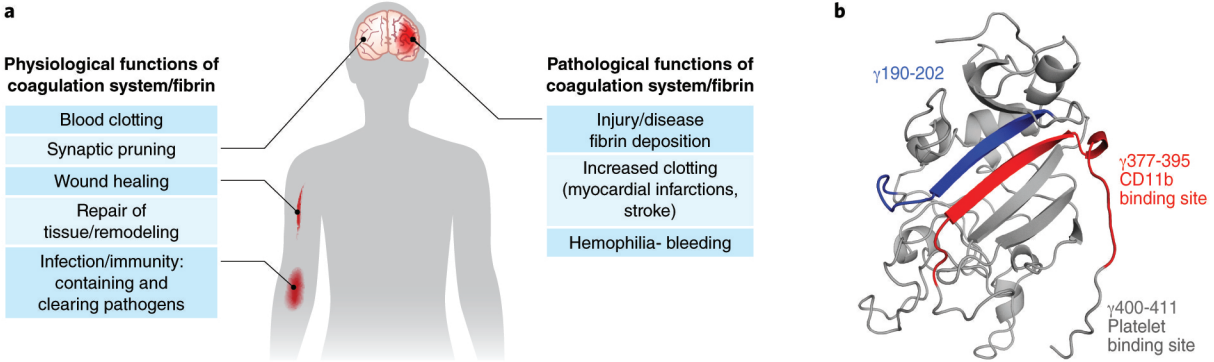
13. Mestas, J. & Hughes, C.C. *J. Immunol.* **172**, 2731–2738 (2004).

14. Takada, Y. *et al*. *J. Autoimmun.* **34**, 453–459 (2010).

15. Luo, D. *et al*. *J. Immunol.* **190**, 4149–4161 (2013).

Figure 1.

The coagulation system and fibrin fulfill pleiotropic roles in biology besides coagulation (**a**, left), and numerous pathologies result from perturbations of their interplay (**a**, right). The ribbon model of the fibrin molecule (**b**) highlights the amino acid positions that are relevant for fibrin polymerization (γ400-411), the ones to which mab 5B8 binds (γ377-395), which blocks interactions with complement receptor 3 and activation of the NADP oxidase pathway as shown by Akassoglou and colleagues[1], and finally another cryptic epitope (γ190-202), which was employed for control purposes in the antibody screening strategy.


a
Physiological functions of coagulation system/fibrin
Blood clotting
Synaptic pruning
Wound healing
Repair of tissue/remodeling
Infection/immunity: containing and clearing pathogens
Pathological functions of coagulation system/fibrin
Injury/disease fibrin deposition
Increased clotting (myocardial infarctions, stroke)
Hemophilia- bleeding
b
γ190-202
γ377-395 CD11b binding site
γ400-411 Platelet binding site